Role of Oxygen Metabolite in the Oxygen Paradox in Hypoxic Myocardium

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=Abstract= The study was conducted to confirm the hypothesis that cytotoxic oxygen free radicals are involved in myocardial cellular damage upon reoxygenation of hypoxic hearts (oxygen paradox), and to identify which species of oxygen radicals might be responsible for the damage.

Oxygen paradox was induced in isolated, Langendorff preparations of rat heart by hypoxic, cardioplegic perfusion (90 min) followed by oxygen-repleted perfusion (20 min). The releases of cytosolic enzymes (creatine phosphokinase, CPK; lactic dehydrogenase, LDH) and a lipid peroxidation product, malondialdehyde (MDA) into the coronary effluent were assayed as the indicators of myocardial cellular damage. To confirm the generation of oxygen radicals during the process of oxygen paradox, \( \text{H}_2\text{O}_2 \) released into the coronary effluent was detected using a fluorescent dichlorofluorescein assay.

The releases of CPK, LDH and MDA into the coronary effluent were abruptly increased by reoxygenation in isolated hypoxic rat hearts. The concentration of \( \text{H}_2\text{O}_2 \) detected in the coronary effluent was also markedly elevated upon reoxygenation. The increases of enzymes and a lipid peroxidation product were prevented to various degrees by scavengers of \( \text{O}_2^- \) (superoxide dismutase, SOD, 10,000 U), \( \text{H}_2\text{O}_2 \) (catalase, 25,000 U) and \( \text{OH}^- \) (dimethylsulfoxide, DMSO, 10%), and by an iron-chelator deferoxamine (0.5 mM). \( \text{H}_2\text{O}_2 \) in the coronary effluent increased dramatically with the administration of SOD in the perfusion solutions, while it was never detected when catalase was given.

It was concluded from these results that the generation of oxygen free radicals increased during the process of oxygen paradox in hypoxic hearts, and that the hydroxyl radical might play a major contributing role in the development of the myocardial cellular damage through the lipid peroxidation of cell membranes.

Key words: Hypoxic myocardium, Oxygen paradox, Oxygen free radical, Lipid peroxidation

INTRODUCTION

It is well known that reoxygenation of a previously hypoxic heart results in significant myocardial damage rather than improvement. This phenomenon has been termed the oxygen paradox (Hearse et al. 1973). The oxygen paradox in experimental animals shares similar pathophysiologic findings with the reperfusion injury which results during reperfusion of ischemic myocardium by coronary by-pass surgery or thrombolytic therapy in human patients (Hess and Manson 1984).

The mechanisms responsible for the cellular damage in the hypoxic–reoxygenated myocardium have not been clearly established. Recently, possible involvement of toxic oxygen radicals (superoxide

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anion, $O^\cdot_2$; hydrogen peroxide, $H_2O_2$; hydroxyl radical, $OH^\cdot$) has been suggested to play a crucial role in the development of myocardial injury in the process of the oxygen paradox. This is supported by observations that exogenous scavengers of oxygen radicals significantly reduce functional and biochemical indices of damage in isolated hypoxic–reoxygenated animal hearts (Gaudel and Duvernoy 1984; Guarnieri et al. 1980; Myers et al. 1985). However, although these studies showed significant cardioprotective effects of oxygen radical scavengers, it was only indirect evidence of oxygen radical involvement, because they did not find the production of oxygen radicals directly in myocardial tissue. Furthermore, these scavenger studies provided little information about which oxygen radical species might be responsible for the myocardial damage. The purpose of the present study was to confirm the oxygen radical generation during the course of oxygen paradox and to identify particular species of oxygen radical which might play a major role in the myocardial damage. For that purpose, $H_2O_2$ released into the coronary effluent was measured by a sensitive fluorometric method using fluorescent dichlorofluorescin diacetate, and cardioprotective effects of various selective exogenous oxygen radical scavengers and other intervention (iron chelator) were reevaluated. The primary indices of myocardial damage were releases of intracellular enzymes (creatine phosphokinase and lactate dehydrogenase) and a lipid peroxidation product, malondialdehyde into the coronary effluent.

**MATERIALS AND METHODS**

Adenosine diphosphate (ADP), adenosine monophosphate (AMP), catalase, creatine phosphate (CP), glucose–6–phosphate dehydrogenase (G–6–PDH), hematin, hexokinase, nicotinamide adenine dinucleotide phosphate (NADP), reduced nicotinamide dinucleotide (NADH), sodium pyruvate, superoxide dismutase (SOD), thiobarbituric acid were purchased from Sigma Chemical Co.. 2′,7′–dichlorofluorescin diacetate (DCFDA) was obtained from Eastman–Kodak, dimethylsulfoxide (DMSO) was from Merck, deferoxamine was from Ciba–Geigy, and the other chemicals were reagent grade.

Isolated heart preparations and induction of oxygen paradox

Sprague–Dawley rats of either sex, weighing 150–200 g were heparinized intraperitoneally (100 iu). Forty-five min after the administration of heparin, the heart was quickly removed and was perfused through the aorta cannulated with a stainless steel cannula attached to a Langendorff perfusion apparatus at a constant perfusion pressure of 100 cm $H_2O$. The perfusion solution was Krebs–Henseleit bicarbonate buffer (K–H solution) containing (in mM) NaCl 118, NaHCO3 27.2, KCl 4.8, MgSO4 • 7H2O 1.2, KH2PO4 1, CaCl2 1.2 and glucose 11.1, and was saturated with a 95% O2–5% CO2 gas mixture yielding a pH value of 7.4 at 37°C. The heart was kept in a humidified chamber maintained at 37°C during the perfusion. The pulmonary artery was incised to allow complete drainage of the coronary effluent. After 20 min of controlled perfusion for wash–out of the residual blood and equilibration, the heart was subjected to 90 min of hypoxic perfusion followed by 20 min of reoxygenated perfusion. Hypoxic and reoxygenated perfusion were done with cardioplegic solution in which K+ concentration had been increased to 15 mM with a corresponding decrease in Na+ concentration. The hypoxic solution in which glucose had been replaced by mannitol (substrate–free solution) was equilibrated with 95% N2–5% CO2. The reoxygenated cardioplegic solution was saturated with 95% O2–5% CO2 gas mixture. The reason why using the cardioplegic solution, instead of normal K–H solution, was to prevent the unexpected release of intracellular enzymes due to the occurrence of irregular spasmmodic beating during hypoxic or reoxygenated period and so to provide a more reproducible experimental condition (Hearse et al. 1973). Some control hearts were perfused with oxygenated K–H solution for 2 hrs. At the end of each experiment, the hearts were removed, and after having bled excess fluid, the tissues were weighed.

**Indices of myocardial damage**

Release of intracellular enzymes, creatine phosphokinase (CPK) and lactic dehydrogenase (LDH) into the coronary effluent was used as indicator of myocardial cellular damage. Coronary effluents were collected at indicated time intervals during hypoxic and reoxygenated perfusion. After measurement of the volume, the samples were kept in ice until the assays were completed (within 8 hrs).

CPK activity was assayed by UV–spectrophotometrically (Bergmeyer 1974). The reaction mixture (pH 6.9) contained imidazole 100 mM, glucose 20 mM, MgCl2 10 mM, ADP 1 mM, AMP 10 mM,
CP 20 mM, NADP 0.7 mM, cysteine HCl 10 mM, hexokinase 0.94 U/ml and G-6-PDH 0.48 U/ml. The reaction was started by the addition of 0.05 ml of coronary effluent sample into 2.95 ml of the reaction mixture, and the rate of change of optical density at 25°C and 340 nm was measured with UV-spectrophotometer (Perkin-Elmer Model 139).

LDH activity was assayed by UV-spectrophotometry (Bergmeyer 1974). 0.5 ml of the sample was added into 2.5 ml of the reaction mixture contained 48 mM phosphate buffer (pH 7.5), 0.6 mM pyruvate and 0.18 mM NADH. The rate of change of optical density was measured by UV-spectrophotometer at 25°C and 340 nm.

The degree of lipid peroxidation in myocardial tissue was also estimated. A lipid peroxidation product, malondialdehyde (MDA) released into the coronary effluent was measured by thiobarbituric acid method (Yagi 1982). 2.4 ml of the coronary effluent was added into 0.6 ml of 1:1 mixture of 0.67% thiobarbituric acid and glacial acetic acid. The reaction mixture was placed on boiling water bath for 60 min and then cooled to room temperature. After cooling, the absorbance was measured at 532 nm with UV-VIS spectrophotometer (Perkin-Elmer Model 139). The MDA released was expressed as nmole/min/g wet wt using the molar extinction coefficient of 1.52 × 10³ M/cm (Placer et al. 1966).

\[ \text{H}_2\text{O}_2 \text{ assay} \]

As measured by \( \text{H}_2\text{O}_2 \) released into the coronary effluent, the production of oxygen radical during the process of oxygen paradox was observed. The concentration of \( \text{H}_2\text{O}_2 \) was assayed spectrophotometrically using 2',7'-dichlorofluorescin diacetate (DCFDA) (Cathcart et al. 1983). 0.2 ml of coronary effluent collected at intervals during hypoxic and reoxygenated periods was mixed with 3 ml of a reaction mixture contained sodium phosphate buffer 25 mM (pH 7.2), DCFDA 0.01 mM and hematin 3.2 µM. The mixture was incubated for 50 min at 50°C. After cooling to room temperature, the fluorescence was measured with spectrophotometer (Perkin-Elmer model 1000) at the excitation wavelength of 452 nm and the emission wavelength of 550 nm. Just prior to the assay, DCFDA was converted to dichlorofluorescein. 0.5 ml of 1 mM DCFDA in ethanol was added to 2.0 ml of 0.01 N NaOH and allowed to stand at room temperature for 30 min. The hydrolysate was then neutralized with 10 ml of 25 mM sodium phosphate buffer (pH 7.2) and stored on ice. The hematin solution was prepared by dissolving 1 mg of hematin in 0.5 ml of 0.2 N NaOH and then diluting to 100 ml with 25 mM sodium phosphate buffer (pH 7.2). This solution was made fresh each day.

**Administration of oxygen radical scavenger**

To assess the possible involvement of oxygen radicals in the development of myocardial damage, cardioprotective effects of exogenous, specific oxygen radical scavengers and other interventions were evaluated. Among the scavengers studied, superoxide dismutase (SOD) which dismutates superoxide anion (\( \text{O}^{2-} \)) enzymatically, and catalase which degrades \( \text{H}_2\text{O}_2 \) were administered through the aortic cannula by use of an infusion pump. The infusion continued for 50 min starting from 60 min of hypoxic period to the end of reoxygenation at a rate of 0.5 ml/min. Total amount of enzymes administered were SOD, 10,000 U and catalase, 25,000 U. A OH⁻ scavenger, dimethylsulfoxide (DMSO) was dissolved in perfusion solution at a final concentration of 10%, and administered for 25 min from 5 min before oxygen repellet to the end of reoxygenation.

To deduce a particular oxygen radical species which may play a major role in the pathogenesis of the oxygen paradox, the effect of a highly specific iron chelator, deferoxamine was also evaluated. Deferoxamine (5 mM) was infused through the aortic cannula at a rate of 0.5 ml/min for 50 min starting from 60 min of hypoxic perfusion to the end of reoxygenated perfusion. It's final concentration in perfusates was about 0.5 mM.

**RESULTS**

**Effects of oxygen radical scavenger and deferoxamine**

**Coronary flow.** In the control hearts that were perfused with oxygenated K-H solution, coronary flow was maintained constant (about 11 ml/min/g wet wt) throughout 2hr perfusion period. Coronary flow changes occurred during hypoxia and oxygen repellet are shown in Table 1. In untreated hearts, coronary flow during hypoxia decreased gradually with time. At 90 min of hypoxia, the flow was 63% of prehypoxic value. Oxygen repellet following 90 min of hypoxia further decreased the flow to 50% of prehypoxic value at the end of reoxygenation period (20 min). However, in the scavenger treated hearts, coronary flows during both hypoxia and reoxygenation, although reduced from prehypoxic values, were considerably greater than those of un-
Table 1. Effects of oxygen radical scavengers on coronary flow during hypoxia and reoxygenation in isolated rat heart

<table>
<thead>
<tr>
<th>Conditions</th>
<th>No. of Animal</th>
<th>Control Perfusion</th>
<th>Hypoxia(min)</th>
<th>Reoxygenation(min)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>60</td>
<td>90</td>
</tr>
<tr>
<td>Untreated</td>
<td>12</td>
<td>11.4±0.6</td>
<td>8.8±0.5</td>
<td>7.2±0.3</td>
</tr>
<tr>
<td>SOD (10,000 U)</td>
<td>7</td>
<td>11.0±1.1</td>
<td>10.1±0.5*</td>
<td>8.4±0.5*</td>
</tr>
<tr>
<td>Catalase (25,000 U)</td>
<td>12</td>
<td>13.1±1.0</td>
<td>10.4±0.5*</td>
<td>7.7±0.5*</td>
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<tr>
<td>DMSO(10%)</td>
<td>5</td>
<td>10.4±0.8</td>
<td>11.7±0.3*</td>
<td>8.5±0.7*</td>
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<td></td>
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<td></td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
<td>6.4±0.2</td>
<td>5.7±0.2</td>
<td>5.6±0.2</td>
</tr>
<tr>
<td>SOD (10,000 U)</td>
<td></td>
<td>7.9±0.3**</td>
<td>7.1±0.4**</td>
<td>7.1±0.4**</td>
</tr>
<tr>
<td>Catalase (25,000 U)</td>
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<td>7.3±0.5*</td>
<td>6.8±0.5*</td>
<td>6.8±0.5*</td>
</tr>
<tr>
<td>DMSO(10%)</td>
<td></td>
<td>8.4±0.4**</td>
<td>8.3±0.4**</td>
<td>7.9±0.5**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td>20</td>
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<tr>
<td>Untreated</td>
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<td></td>
</tr>
<tr>
<td>SOD (10,000 U)</td>
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<tr>
<td>Catalase (25,000 U)</td>
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<td></td>
</tr>
<tr>
<td>DMSO(10%)</td>
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*p < 0.05, **p < 0.01

Fig. 1. Effects of oxygen radical scavengers and deferoxamine on creatine phosphokinase release in hypoxic-reoxygenated rat heart. Isolated rat hearts were subjected to 90 min of hypoxic, substrate-free perfusion followed by 20 min of oxygen-repleted perfusion. Superoxide dismutase(SOD, 10,000 U), catalase(25,000 U) or deferoxamine(0.5 mM) were infused for 50 min, from 30 min before reoxygenation to the end of perfusion. Dimethylsulfoxide (DMSO, 10%) dissolved in perfusion medium was administered from 5 min before reoxygenation and throughout the reoxygenation period.

Untreated treated hearts. At the end of reoxygenation the flows increased by 5-25% compared to corresponding untreated flows. Oxygen radical scavengers at concentrations used did not change the coronary flow in the oxygenated control hearts(data not shown).

Enzyme release. Release of intracellular enzymes during hypoxia was not so significant. After 90 min of hypoxia, the rate of CPK release into the coronary effluent was 0.08 U/min/g wet wt. However, in the hearts subjected to hypoxia followed by reoxygenation, there was a sudden, massive enzyme release upon oxygen repletion. As in Fig. 1, the rate of CPK release was abruptly increased to a maximum of 4.6 U/min/g wet wt at 2 min of reoxygenation period, which was a 58 fold increase compared to that of hypoxia. This increased rate was suppressed significantly by SOD, catalase or DMSO. In addition to those scavengers, a specific Fe^{+++}-chelator, deferoxamine also caused a sig-
nificant reduction of CPK release upon reoxygenation.

In LDH(Fig. 2), the rate of release during hypoxia was 0.1 U/min/g wet wt(at 90 min), and it was suddenly increased by a maximum of 21-fold at 2 min of reoxygenation. Oxygen radical scavengers or deferoxamine also suppressed the LDH release significantly during reoxygenation.

The addition of oxygen radical scavengers or deferoxamine to the perfusing solutions neither induced nor decreased the intracellular enzyme release in the control heart preparations perfused with oxygenated K-H solution for 2 hrs (data not shown).

Lipid peroxidation. The degree of lipid peroxidation in the myocardial tissue was estimated from the release of MDA into the coronary effluent. During 90 min of hypoxia, MDA was not detected in the coronary effluent. However, in the heart preparations subjected to 90 min of hypoxia followed by 20 min of reoxygenation, MDA was abruptly released. The maximum rate of release was 2.76 n mole/g wet wt at 3 min of reoxygenated perfusion(Fig. 3). This MDA release was prevented significantly in the presence of oxygen radical scavengers or deferoxamine. SOD, catalase, DMSO or deferoxamine did not induce the MDA release in the control hearts (data not shown).

Fig. 3. Effects of oxygen radical scavengers and deferoxamine on lipid peroxidation in hypoxic-reoxygenated rat heart. Lipid peroxidation of myocardial tissue was estimated from malondialdehyde release assayed by thiobarbituric method. Perfusion conditions and methods of scavenger administration were same as in Fig. 1.

Fig. 4. H$_2$O$_2$ production during reoxygenation in hypoxic rat heart. Isolated rat hearts were subjected to 90 min of hypoxic, substrate-free perfusion followed by 20 min of oxygen-repleted perfusion. H$_2$O$_2$ released into the coronary effluent during reoxygenation period was estimated by fluorescent dichlorofluorescein assay method.

H$_2$O$_2$ production

To confirm the generation of oxygen radical during the process of the oxygen paradox, H$_2$O$_2$ in the coronary effluent was measured. H$_2$O$_2$ was abruptly increased with oxygen repletion in the isolated, hypoxic rat heart preparations. The rate of H$_2$O$_2$ production during hypoxia was 0.2 nmole/min/g wet wt (at 90 min), and it was suddenly increased to a maximum value of 2.62 nmole/min/g wet wt by reoxygenation(at 2 min), which was a 13 fold increase compared to hypoxic value(Fig. 4). This H$_2$O$_2$ production was similar in pattern with the release of intracellular enzymes or MDA. Although H$_2$O$_2$ was never detected in the coronary effluent in the presence of catalase, it was greatly increased with the addition of SOD in the perfusing solution. The rate of H$_2$O$_2$ production in the presence of SOD was 13.25 nmole/min/g wet wt during hypoxia(at 90 min) and 24.5 nmole/min/g wet wt upon oxygen repletion(at 1 min)(Table 2).

DISCUSSION

Although different possible mechanisms which have been reported as contributing factors for the myocardial damage during the process of oxygen paradox include depletion of intracellular high energy pool(Jennings and Reimer 1981), release of endogenous catecholamines (Gaudel et al.
Table 2. Effects of superoxide dismutase (SOD) and catalase on H$_2$O$_2$ production in hypoxic–reoxygenated, isolated rat heart

<table>
<thead>
<tr>
<th>Conditions</th>
<th>No. of Animal</th>
<th>Hypoxia(min)</th>
<th>H$_2$O$_2$ production(nmole/min/g wet wt.)</th>
<th>Reoxygenation(min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>90</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Untreated</td>
<td>11</td>
<td>0.2</td>
<td>1.82</td>
<td>2.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 0.09</td>
<td>±0.38</td>
<td>±0.20</td>
</tr>
<tr>
<td>SOD (10,000 U)</td>
<td>6</td>
<td>13.25</td>
<td>24.51</td>
<td>19.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 1.31</td>
<td>±0.05</td>
<td>±1.98</td>
</tr>
<tr>
<td>Catalase</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(25,000 U)</td>
<td></td>
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1979), excessive intracellular calcium accumulations (Katz and Reuter 1979), activation of phospholipases (Chien et al. 1981) and accumulation of lysophosphatidic (Pitts & Okhuysen 1984), there is no known single most probable theory.

Recently, possible involvement of toxic oxygen free radicals has been also suggested to play a crucial role in the development of myocardial damage that occurs during reoxygenation of hypoxic myocardium (Gaudel and Duvelloery 1984; Guarneri et al. 1980; Myers et al. 1985). Studies with isolated myocardial tissue in vitro indicate that hypoxia or ischemia causes an increase in intracellular reducing equivalents, a disturbance of mitochondrial electron transport, the conversion of xanthine dehydrogenase to xanthine oxidase, and an accumulation of ATP breakdown products that act as substrate for oxygen radical production through the action of xanthine oxidase. During reoxygenation of the hypoxic tissue, all of the above conditions favor a burst of oxygen radical production (Fridovich 1978; Hess and Manson 1984; Meerson et al. 1982). In the present study, the release of CPK or LDH into the coronary effluent was abruptly increased by reoxygenation in isolated hypoxic rat hearts, and this increase was suppressed to various degrees by scavengers of O$_2^*$, H$_2$O$_2$ or OH·. This result supported the hypothesis that toxic oxygen radicals were involved with the pathogenesis of the oxygen paradox in myocardial tissue.

However, this cardioprotective effect of the scavengers observed in this study and other previous reports (Gaudel and Duvelloery 1984; Myers et al. 1985) provided only indirect evidence of oxygen radical involvement. If the toxic oxygen radicals indeed mediate the phenomenon of oxygen paradox, the radicals should be detected in myocardial tissue during the course of oxygen paradox. Using electron spectroscopy, Rao and Muller (1983) found an increase in the production of a certain kind of free radical in ischemic–reperfused tissue, but oxygen radicals have never been detected directly in myocardial tissue. Because O$_2^*$ and OH· exist transiently in aqueous environment, it is very difficult to measure the production of these radicals in tissues by presently available analytical methods indebted to their oxidation–reduction activities. A relatively stable species, H$_2$O$_2$, however, could be measured in biological materials by a sensitive fluorimetric method using fluorescent dichlorofluorescin (Cathcart 1983; Homan-Müller et al. 1975). Homan-Müller and colleagues (1975) quantitated H$_2$O$_2$ produced by phagocytizing human granulocytes. In this study, H$_2$O$_2$ was detected in the coronary effluent and it was massively increased upon reoxygenation. This H$_2$O$_2$ increase was comparable to the enzyme release. Furthermore, the H$_2$O$_2$ production was more pronounced with the administration of SOD in the perfusion solution. Considering that SOD catalyzes dismutation reaction converting O$_2^*$ to H$_2$O$_2$(O$_2^* + O_2 + 2H^+ \rightarrow 2$H$_2$O$_2^*$), this result seemed to be a reflection of increased production of O$_2^*$. By comparison with some of the other oxygen species, O$_2^*$ and H$_2$O$_2$ are not very reactive in biological systems. However, in several biological systems O$_2^*$ and H$_2$O$_2$ have been implicated as precursors for another reactive species, OH·. As a mechanism of production of OH·, iron-catalyzed Haber-Weiss reaction (O$_2^*$ + Fe$^{+++}$ $\rightarrow$ O$_2$ + Fe$^{++}$, H$_2$O$_2$ + Fe$^{++}$ $\rightarrow$ OH· + OH$^-$ + Fe$^{+++}$) has been proposed (Fong et al. 1976; Fridovich 1978; Halliwell 1978). The significant reduction of intracellular enzyme and MDA release not only by
SOD, catalase or DMSO, but also by deferoxamine, a highly specific Fe^{3+} chelator, may therefore be explained in terms of suppression of OH\cdot formation. It was supported by the observations that OH\cdot inhibited calcium binding of cardiac sarcoplasmic reticulum in vitro reaction conditions containing oxygen radical generating system(Kim et al. 1984), and that deferoxamine suppressed inflammation in several animal models and inhibited iron-catalyzed lipid peroxidation in vitro(Blake et al. 1983).

The result presented in this paper indicated that the peroxidation of membrane lipids, estimated from MDA production had been triggered by oxygen radicals. This finding agrees with the studies of other investigators(Gaudel and Duvelleroy 1984; Guarnieri et al. 1980; Kim and Akera 1987) who reported the prevention of MDA production by oxygen radical scavengers in hypoxic-reoxygenated hearts. The lipid peroxidation and the resultant biochemical changes may alter physical properties of membranes, such as changes in lipid microenvironments of membrane bound enzymes and also increase in membrane permeability (Freeman and Crapo, 1982). This fact was confirmed by the close relation which existed between the rate of intracellular enzymes and MDA releases. In the present study, MDA released into the coronary effluent, instead of its sarcosomal content were measured, due to the analytical difficulties. The results, therefore, do not specifically show the degree of sarcosomal lipid peroxidation. However, it should be pointed out that sarcosomal membranes has been shown to be highly susceptible to oxygen radical-induced lipid peroxidation compared with lipid bilayers of other cellular components(Kramer et al. 1984). Therefore, the myocardial damage in hypoxic-reoxygenated cardiac tissue will be largely dependent on the lipid peroxidation in cell membranes by oxygen radical.

In summary, the present study provides further evidence of the involvement of toxic oxygen radicals, particularly hydroxyl radical (OH\cdot), in the pathogenesis of myocardial damage in the oxygen paradox.

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지산소 심장의 oxygen paradox시 산소대사물의 역할

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지산소 심근의 산소제공군에 따른 심근손상(oxygen paradox)에 있어서 유독성 산소대사물인 산소라디칼의 관현성을 확인하고, 심근손상을 이르는 데 주로 관여하는 타지질이 무엇인지를 정의하였다. Langendorff 관류장치를 사용한 치료적심장에서 실험적 oxygen paradox를 유도하였으며, 심근손상의 차로에 세포질 효소인 creatine phosphokinase(CPK), lactic dehydrogenase(LDH), 및 저감파산화 산물의 하나인 malondialdehyde(MDA)의 활성관목액으로의 유출을 측정하였다. 산소라디칼의 생성을 확인하기 위하여 역시 관성관목액중의 H_{2}O_{2}를 형성하는 dichlorofluorescin을 이용한 fluorescence법으로 측정하였다. 지산소관류(90분)에 이은 산소제공급관류(20분)시 CPK, LDH 및 MDA의 활성액으로의 유출이 산소 제공금과 더불어 급격히 증가하였으며, H_{2}O_{2} 또한 산소 제공금시 현저히 증가하였다. O_{2}^{=} 제거효소인 superoxide dismutase(SOD, 10,000 U), H_{2}O_{2} 분해 효소인 catalase(25,000 U), OH^{=} 제거물질인 dimethylsulfoxide(DMSO 10%), 천이온 칼리아트제도의 deferoxamine(0.5 mM)은 CPK, LDH 및 MDA 유리를 억제하였다. 관성관목액중의 H_{2}O_{2}는 catalase 두여시에는 전혀 측정되지 않았으나, SOD 두여에 의하여는 오히려 더욱 현저한 증가를 보였다. 이상의 실험에서 치료적심장의 oxygen paradox시 산소라디칼의 생성 증가를 확인하였으며, 산소라디칼 중에서는 OH^{=}이 심근손상을 유발하는 데 주로 관여할 것으로 결론지었다.